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Authors: Banish, Linda D., and Gilmartin, William G.

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HEMATOLOGY AND SERUM CHEMISTRY OF THE YOUNG HAWAIIAN MONK SEAL (*MONACHUS SCHAUINSLANDI*)

Linda D. Banish and William G. Gilmartin
Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service,
National Oceanic and Atmospheric Administration, 2570 Dole Street,
Honolulu, Hawaii 96822, USA

ABSTRACT: Between January 1984 and May 1987, blood samples were collected from 12 young (3- to 6-mo-old) Hawaiian monk seals (*Monachus schauinslandi*) that were captured in the wild and held in captivity. All samples evaluated were from clinically normal animals. Average hematologic and serum chemistry values were not remarkable for a young diving mammal. The blood and serum analyses performed established reference ranges, which can be used as indicators of health status for this endangered species.

Key words: Hawaiian monk seal, *Monachus schauinslandi*, hematology, serum chemistry, reference ranges.

INTRODUCTION

The breeding range of the endangered Hawaiian monk seal (*Monachus schauinslandi*) is limited to the northwestern Hawaiian Islands, from Nihoa Island to Kure Atoll in the Hawaiian Archipelago. In the past, human disturbance has been responsible for significant population declines (Kenyon and Rice, 1959; Rice, 1960; Johnson et al., 1982). Other environmental factors also may limit the size of the population: shark predation (Taylor and Naftel, 1978; Balazs and Whittow, 1979; Alcorn and Kam, 1986), natural toxins (Gilmartin et al., 1980), injury of adult females and immature seals of both sexes from mobbing by adult male seals (Johnson and Johnson, 1981; Alcorn, 1984), entanglement in debris (Henderson, 1984), and food availability (Kenyon, 1973). Total seal beach counts over the breeding range have been reduced to <50% of the counts observed in the late 1950's.

Research activities to assist recovery of the Hawaiian monk seal population currently involve the use of animals held in temporary and permanent captivity. Reference ranges for normal hematologic and serum chemistry values are needed for routine clinical evaluation of all captive animals. For young animals in temporary captivity, indices are useful also as part of a screening program (performed prior to release), which protects against the potential transfer of disease between island populations.

MATERIALS AND METHODS

General methods

In 1984–1987, 12 recently weaned Hawaiian monk seals were collected from the northwestern Hawaiian Islands for various research activities in Honolulu. Hematology and serum chemistry analyses were performed on each animal at least once while between 3 and 6 mo of age.

In captivity, the young seals were fed a diet of frozen herring and smelt at 5–8% of their body weight and were supplemented with multivitamins, thiamine, and vitamin E. They were housed in enclosures that allowed swimming and haul-out activities.

Animals were not anesthetized for samplings and were manually restrained. Efforts were made to minimize the stress of handling, and seals were cooled with water throughout the procedures. An 18-gauge, 7.6-cm spinal needle and multiple 35-cc plastic syringes were used to draw 60–70 cc of whole blood from the extradural vein in the caudal lumbar area as described in Geraci and Smith (1975). Silicone-treated glass collection tubes were used for serum collection, and tubes containing ethylenediaminetetraacetic acid disodium salt (Na-EDTA) were used for hemogram studies. Fresh blood smears were prepared on glass slides for differential counts of white blood cells. All samples were centrifuged and separated within 1 hr of collection. Serum samples were refrigerated between the time of blood separation and serum analysis.

In Hawaii, available laboratory resources for

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blood analyses are limited to automated techniques that are set to human standards. Delivery of samples to a marine mammal reference laboratory requires a minimum of 2 days transit time and is impractical for the clinical purposes of the Hawaiian monk seal program on Oahu. All hematology and serum chemistry analyses used in computations of reference ranges were performed by Accupath, a SmithKline Bioscience Laboratory (Honolulu, Hawaii 96822, USA). Coulter 4C hematology reference control (Coulter Electronics, Inc., Hialeah, Florida 33010, USA) was used for all hematological determinations, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), and mean corpuscular hemoglobin concentration (MCHC). At least 100 leukocytes were counted to determine differential cell counts on Wright-Giemsa-stained smears.

Blood chemistry tests performed on the Boehringer Mannheim Diagnostics 8600R analyzer (Boehringer Mannheim Diagnostics Division, 9115 Hague Road, Indianapolis, Indiana 46250, USA) and respective methods included total protein, Biuret; albumin, Bromcresol green; glucose, hexokinase; blood urea nitrogen, UV endpoint; creatinine, Jaffe; uric acid, uricase; bilirubin, Jendrassik-Groff (modified); cholesterol, enzymatic; triglycerides, enzymatic; alkaline phosphatase, alpha-napthyl phosphate; aspartate aminotransferase and alanine aminotransferase, IFCC; lactate dehydrogenase, Wacker (modified); gamma glutamyl transferase, Szasz + Persign; sodium and potassium, flame photometry; chloride, Schoenfeld + Lewellen (modified); calcium, Cresophthalein compexone; and phosphorous, Phosphomolybdate. Globulin values were calculated by subtracting albumin from total protein.

The data sets for each of the hematologic and serum chemistry parameters were examined for normal distribution by the Shapiro-Wilk statistic (SAS Institute Inc., 1985). The mean ($\bar{x}$), median, and standard deviation (SD) were determined by Grubbs T-statistic (Grubbs, 1969; Lumsden and Mullen, 1978). Statistics reported for data sets were then recalculated with "outliers" removed.

Verification of laboratory methods

As is well known, differences in analytical equipment and methodologies as well as use of differing reference standards can lead to variable results between analytical laboratories (International Symposium on Quality Control 1979; Hector, 1986; Blijenberg et al., 1987). To establish the validity of measured values as determined by the local human reference laboratory (Accupath) in relation to those that might be determined at a facility with machines standardized for pinniped studies, four sets of whole blood and serum samples were split for duplicate analyses by Accupath and the Animal Care Laboratory at Sea World (San Diego, California 92109, USA). Both whole blood and serum analyses were performed at Accupath on the day of animal sampling and again on the date of evaluation by the Animal Care Laboratory.

Sodium heparin has been shown to be the anticoagulant most effective at stabilizing whole blood during long storage (Geraci and Engelhardt, 1974). Whole blood stored in EDTA at 4 C for 2 days should not be significantly altered by time-related changes (Geraci and Engelhardt, 1974). To insure that the type of anticoagulant used was not a limiting factor, the hemogram samples sent to Sea World for analysis were again split for storage in both sodium heparin and EDTA. Differences between paired whole blood and serum samples were evaluated by a two-tailed Student's t-test (Weissberg and Beatty, 1960).

RESULTS

Hematologic and serum chemistry data are summarized and presented in Tables 1 and 2. Data sets were normally distributed for all but two parameters, basophils and band neutrophils (skewness: bands, 2.08; basophils, 2.45). Of 16 white blood cell differential counts, only four animals exhibited band neutrophils (5%, 4%, 2%, 1%), and only two animals, basophils (1%, 1%). These two parameters were not treated for outliers because of skew.

No outliers were found among the other individual hematologic measurements. Among the serum chemistry values, only one outlier was found and was excluded from statistical determinations: alkaline phosphatase was elevated in one seal at 521 U/liter. There were no apparent indications of a pathologic or iatrogenic cause for elevated alkaline phosphatase in this animal.

For the test of validation of hematologic data, the paired blood samples analyzed at the Sea World laboratory did not differ significantly ($P = 0.05$) from those values reported by Accupath. Neither were there

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TABLE 1. Hematologic values of the Hawaiian monk seals.[a]

Hematologic parameter[b]	n	Range	Mean	Median	SD	$\bar{x} \pm 2$ SD
WBC ($\times 10^3/\mu l$)	16	5.7–11.2	8.8	8.9	1.6	5.6–12.0
Segmented neutrophils (%)	16	2.9–6.05	4.3	4.4	1.0	2.3–6.3
Bands (%)	16	0–0.5	0.1	0	0.2	0–0.41
Lymphocytes (%)	16	1.7–4.6	3.6	3.7	0.9	1.8–5.4
Monocytes (%)	16	0.2–1.1	0.5	0.5	0.3	0–1.1
Eosinophils (%)	16	0–0.6	0.2	0.2	0.2	0–0.5
Basophils (%)	16	0–0.1	0	0	0	0–<0.1
RBC ($\times 10^6/\mu l$)	15	3.1–4.3	3.6	3.7	0.3	3.0–4.2
Hgb (g/dl)	15	14.5–21.8	18.5	18.5	1.7	15.1–21.9
Hct (%)	15	44.2–61.3	54.7	54.8	4.6	45.5–63.9
MCV (fl)	15	137.3–167.0	150.7	150.0	7.6	135.5–165.9
MCH (pg)	15	47.1–54.4	50.9	50.9	2.2	46.5–55.3
MCHC (g/dl)	15	32.6–35.5	33.7	33.8	0.7	32.3–35.1

[a] Analysis performed by Accupath Laboratory.
[b] Key: WBC = white blood cells, RBC = red blood cells, Hgb = hemoglobin, Hct = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

differences between EDTA and heparin storage for the values of hematology samples evaluated at Sea World. Differences between most paired serum chemistry values also did not differ significantly ($P = 0.05$) between the two laboratories. The serum values for the enzyme lactate dehydrogenase and triglyceride were consistently different in all four paired serum samples. Evaluations of three other serum enzymes (alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transferase) differed significantly ($P = 0.05$) between laboratories.

TABLE 2. Serum chemistry values of young Hawaiian monk seals.[a]

Parameter	n	Range	Mean	Median	SD	$\bar{x} \pm 2$ SD
Total protein (g/dl)	20	5.8–8.1	7.0	7.1	0.6	5.8–8.32
Albumin (g/dl)	16	2.4–3.9	3.4	3.5	0.4	2.6–4.2
Globulin (g/dl)	16	3.0–4.5	3.6	3.5	0.5	2.6–4.6
Glucose (mg/dl)	16	88–133	112.1	115	13.3	85.5–138.7
Blood urea nitrogen (mg/dl)	16	9–34	23.6	24.5	6.7	10.2–37.0
Creatinine (mg/dl)	16	0.5–1.7	1.0	1.1	0.3	0.4–1.6
Uric acid (mg/dl)	16	0.8–2.3	1.5	1.5	0.4	0.7–2.3
Total bilirubin (mg/dl)	16	0.2–0.5	0.3	0.3	0.1	0.1–0.5
Direct bilirubin (mg/dl)	16	0–0.1	0.07	0.10	0.05	0–0.17
Cholesterol (mg/dl)	16	187–568	317.3	303	98.4	120.5–514.1
Triglycerides (mg/dl)	16	28–91	55.4	49.5	17.3	20.8–90.0
Alkaline phosphatase (U/liter)	16	75–521	186.2	160.0	94.8	0–375.8
Aspartate aminotransferase (U/liter)	16	9–163	79.5	82.0	50.4	0–180.3
Alanine aminotransferase (U/liter)	16	13–148	58.5	34.0	47.4	0–153.3
Lactate dehydrogenase (U/liter)	16	442–1,544	832	792.5	305.4	221.2–1,442.8
Gamma glutamyl transferase (U/liter)	16	2–13	7.9	8.5	3.6	0.7–15.1
Sodium (meq/liter)	16	150–164	155	155	3.7	147.6–162.4
Potassium (meq/liter)	16	4.7–6.3	5.5	5.5	0.5	4.5–6.5
Chloride (meq/liter)	16	103–118	106.8	105	3.9	99.0–114.6
Calcium (mg/dl)	16	9.4–12.1	11.0	11.1	0.8	9.4–12.5
Phosphorous (mg/dl)	16	4.3–9.3	7.1	6.8	1.5	4.1–10.1

[a] Analysis performed by Accupath Laboratory.

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DISCUSSION

Hematologic adaptations to the aquatic lifestyle of pinnipeds have been examined by several investigators (Bryden and Lim, 1969; Lenfant et al., 1969, 1970; Ronald et al., 1969; Geraci, 1971; Greenwood et al., 1971; Lane et al., 1972; Wells, 1978; Wolk and Kosygin, 1979). Likewise, many comparative hematologic and/or serum chemistry studies between pinniped species have been presented (Lane et al., 1972; Hawkey, 1975; Engelhardt, 1979; Wolk and Kosygin, 1979; Needham et al., 1980). Although the hematologic and serum chemistry values of the young Hawaiian monk seal are not unexpected relative to other diving marine mammals, the purpose of this study is to present clinically useful information on the hematology and serum chemistry of this endangered species, rather than to discuss its physiology in relation to these values or to compare these values to those of other species.

Because of the constraints of dealing with an endangered pinniped species, the sample number is small, and only data collected from animals carefully judged to be clinically healthy are used in this report. Determinants of health status included historical condition, general appearance, activity level, feed intake around the day of sampling, and lack of recognizable disease indicators subsequent to sampling. The reported values are intended to serve as reference values for the clinical evaluation of animals and for recognition and analysis of disease states.

The effect of restraint on hematologic values was not determined. However, the hematologic changes that may occur with the stress response, including neutrophilia, monocytosis, lymphopenia, and eosinopenia (Geraci and Smith, 1975; Duncan and Prasse, 1981), were not notable in subsequent work in which samples were taken from healthy animals sedated by orally administered diazepam prior to venipuncture (Banish, unpubl. data).

Serum chemistry values can vary substantially for the same sample when analyzed by different equipment or methods or both (International Symposium on Quality Control, 1979; Hector, 1986). This is true especially for serum enzymes and was borne out by the paired serum sample analyses of this study. The only parameters in which reported values differed significantly between laboratories were four serum enzymes and triglycerides. Especially marked and consistent differences existed between the two laboratories in the evaluation of lactate dehydrogenase (LDH) and triglycerides. The results reported by the Sea World laboratory were 50% higher for LDH and 50% lower for triglycerides than those of the Accupath laboratory. The values for three other serum enzymes analyzed, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT), were also disparate between the laboratories. In these cases, variations between paired samples were not consistent, but AST averaged 15% lower, ALT 27% lower, and GGT 50% lower at the Sea World facility than at the Accupath laboratory.

Laboratory methods at the Sea World laboratory included the use of Gilford analyses reagents (Ciba-Corning Diagnostics Corp., Gilford Systems, Oberland, Ohio 44074, USA). Triglycerides were evaluated by the Whlefeld method, LDH by the Wacker (modified), ALT and AST by I.F.F.C., and GGT by the Schasz procedure (modified). Despite the differences between laboratories, no values determined by the Sea World laboratory were beyond two standard deviations of the means (Table 2).

This information is clinically instructive in that it reiterates the need to establish a set of "normal" or reference values at a specific laboratory, and the value of using a consistent set of diagnostic laboratory methodologies when using blood data to evaluate any individual relative to a group of its species. At least small differences will likely exist between any two laboratories. Despite differences between the serum

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evaluations at the two laboratories in the test of paired serums, values as reported by this study for all parameters should be useful as indicators of the health status of an individual Hawaiian monk seal at any facility.

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